Research Article
Volume 11:1, 2020
DOI: 10.37421/jch.2020.11.551

Journal of
Cytology & Histology

ISSN: 2157-7099 Open Access

Histological Demonstration of the Organisms Causing Human Tungiasis in Eastern Uganda

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Abstract

Background: Tungiasis, a neglected tropical ecto-parasitic disease, has resurged in Sub-Saharan Africa, causing public concern and at times confusing diagnosis. In October 2010, following widespread human disease within the Busoga sub-region of Eastern Uganda the Ministry of Health sought to verify the cause. Tungal extraction was therefore performed to provide specimens for diagnosis.

Aim: To identify the organisms enucleated from the feet of residents in two affected districts.

Method: The formalin-preserved enucleate was macroscopically described, processed and embedded in paraffin wax. Sections four micrometers thick were then stained with haematoxylin and eosin and microscopically examined.

Results: Histology showed cystic bodies with internal structures. At the periphery a multi-layered cuticle overlay a stratum of hypodermal cells. At the centre, distended globular sections lined by columnar cells characteristic of digestive epithelia had speckled content representing ingested human blood. Eccentric bipolar sections had convoluted microvillous epithelia typical of filtration-excretory surfaces. Eosinophilic rings formed sub-cuticular chains and central clusters, describing tracheal routes. Numerous eosinophilic anisocytic spheres were enclosed in circular sections lined by cuboid cells characteristic of ovarian epithelia. Fat globules and striated muscle were noted; the head, thorax and terminal abdomen were not seen.

Conclusion: The structures described were distinct from those observed in cutaneous helminthiasis, cutaneous myiasis and acariasis. The organisms were thus reported as tissue-embedding haematophagous oviparous arthropods of the genus *Tunga*. Tungiasis has since been listed among the thirteen neglected tropical diseases of highest public health importance in Uganda.

Keywords: Tungiasis • Histology • Insect morphology • Uganda

Introduction

Tungiasis or sand flea disease causes morbidity in African, South American, Caribbean and Asian populations [1,2] and increasingly affects travellers from non-endemic countries [3,4]. Two species *Tunga penetrans* (Linnaeus, 1758) and *T. trimamillata* [5] infect humans. The former afflicts rural and periurban communities in East, Central and West Africa causing public concern, sometimes disgrace and occasionally misdiagnosis [6]. Following media reports of heavy infection and deaths due to sand flea disease in October 2010, the Ministry of Health (MoH) conducted an investigation within the Busoga sub-Region in Eastern Uganda. Severe infection was noted in rural and slum areas, particularly where human dwelling had non-cemented floors and where living space was shared with livestock [7]. Tungal extraction was then performed to relieve heavy infection and to provide specimens for confirmation of diagnosis. In this article we report the histological findings on the material that was submitted for verification of the causative organisms.

Materials and Methods

From the 15th to the 19th of October 2010 material was enucleated from the feet of fifteen heavily infected residents of Jinja and Iganga Districts in the Busoga sub-Region of eastern Uganda. The skin was washed with soap and disinfected using iodine. A sterile needle was then used per individual to scrape the epidermal opening, keratin layer or crust and to extricate the underlying body. Remnant ulcers were cleaned, dressed and referral made to the nearest Health Centre for antibiotic and anti-tetanus coverage. The enucleate was preserved in 10% formalin. In the laboratory the specimens were macroscopically described, processed by automation through a series of graded alcohol 40% to 95%, xylene and molten paraffin before embedment in paraffin wax. Sections cut at four micrometers using a microtome were layered on glass slides, deparaffinised in xylene, rehydrated in a series of graded alcohol 90% to 80% then stained using haematoxylin and counterstained with 1% eosin. The slides were covered using DPX mounting medium and microscopically examined.

Results

The submitted material was grossly described as white cystic tissues 5 to 7 millimetres in diameter, neither head nor excretory-copulatory structures were distinguishable. Microscopy revealed circular bodies with integument that enclosed a haemocoel containing longitudinal, tangential and cross-sections through the internal organs as shown in Figure 1. At the periphery, the non-cellular multilayered epicuticle and procuticle both overlay a stratum of vacuolated externally ciliated hypodermal cells resting on a basal layer and forming the exoskeletal matrix as shown in Figure 2. At the centre were distended globular and elongate sections lined by columnar cells characteristic of digestive epithelia as shown in Figure 3, their homogenous luminal content had lobular structures identical to the nuclei of polymorphonuclear and mononuclear leukocytes in human blood as shown in Figure 4. Eccentric bipolar sections with prominent villous and microvillous epithelium indicated the tubular filtration-excretory Malpighian system as shown in Figure 5. Numerous eosinophilic rings around clear space, representing tracheal trunks, tubes

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Received 14 January 2020; **Accepted** 05 February 2020; **Published** 12 February 2020

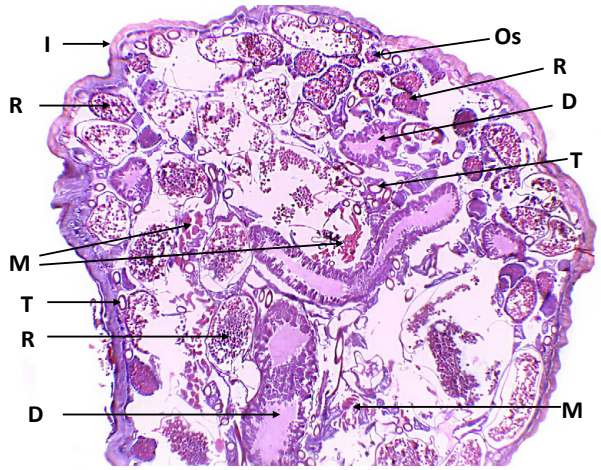

Figure 1. A cross-section through the enucleate from the foot of a person (X 4). The abdomen of a female sand flea, with arrowed integument (I) enclosing the celom containing digestive (D), osmoregulatory (Os), reproductive (R), tracheal (T) and muscle (M) sections.

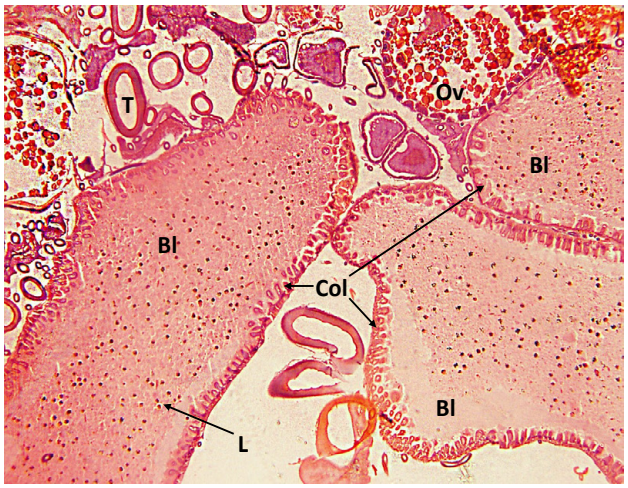

Figure 4. Blood leukocytes within the digestive tract of a gravid female sand flea (X 10). Indicated are the columnar epithelium (Col), ingested blood (Bl) with many leukocytes (L), trachea (T) and ova (Ov).

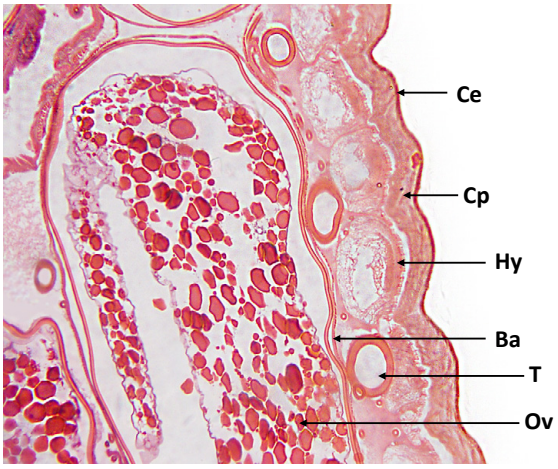

Figure 2. The exoskeletal and sub-cuticular respiratory structures of a hypertrophied female sand flea (X 40). Arrowed are the epicuticle (Ce), procuticle (Cp), hypodermal cell (Hy), basal layer (Ba), trachea (T) and ova (Ov).

Figure 5. The filtration-excretory Malpighian structures in a gravid female sand flea (X 10). Indicated are the villous epithelium with microvilli (V), trachea (T) and ova (Ov).

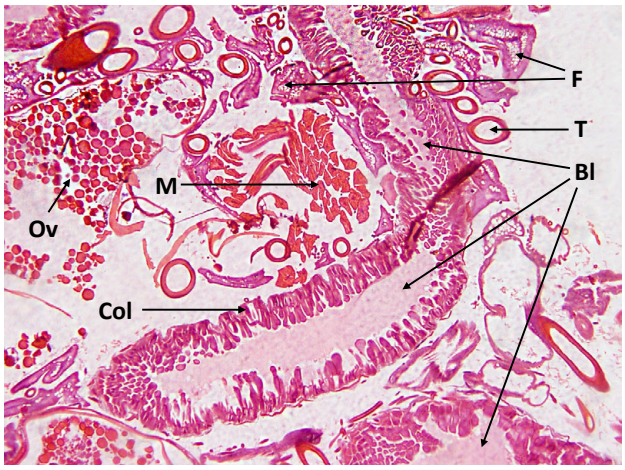

Figure 3. The engorged midgut of a gravid female sand flea (X 20). Arrowed are the columnar epithelium (Col), ingested blood (Bl), muscle (M), fat (F), trachea (T) and ova (Ov).

Figure 6. Ovarian structures in a gravid female sand flea (X 20). Indicated are the cuboid cells (Cu), nucleus (N), basal layer (Ba) and ova (Ov).

and tubules, formed sub-cuticular chains and clusters adjacent to the central structures as shown in Figures 1-6. Equally abundant and conspicuous were the aggregates of eosinophilic micro- and macrocytic spheres indicating ova, which were enclosed in circular and elongate sections as shown in Figures 1-6. The sections were lined by cuboid cells, characteristic of ovarian epithelia, which had large central nuclei and rested on a basal layer as shown in Figure 6. Scattered sections had fat globules; central and peripheral truncated bands of striated muscle were noted as shown in Figures 1 and 3. The head, thorax and terminal abdomen were not seen.

Discussion

Enucleated organisms were embedded in paraffin wax to demonstrate their microscopic features, distinguish them from other ectoparasites and to provide a definite diagnosis. These were female sand fleas, whose chitinous membrane between the second and third abdominal tergal and sternal segments had expanded and hypertrophied thus accommodating ovaries filled with ova at different stages of development and mid-gut engorged with host blood [8,9]. The large number of leukocytes within the ingested blood implied an inflammation induced by the sand flea at the site of embedment, a reaction anticipated following penetration of a foreign body. Biological activity within the organism was inferred by the prominent hypodermis, prolific respiratory system and outstanding filtration-excretory structures, the fat globules were possibly of metabolic and storage significance [10]. A gravid female sand flea expels eggs and faeces. The bands of muscle known to aid this evacuation also attach the head and thorax to the distal abdomen and provide internal support to the flea's inflated abdomen [9]. Their central location distinguished the organism from tissue nematodes whose muscle arranges below the hypodermis, furthermore nematodes do not possess a tracheal system [11].

The microscopic features of a gravid female sand flea were well described for *T. penetrans* which was harvested from inhabitants of Congo Brazzaville, Belgian Congo and Puerto Rico; for *T. penetrans* embedded in human tissue and for *T. trimamillata* extracted from cattle [12]. In our examination however, no cuticular extensions suggestive of head or ano-genital structures were seen. These may have detached during enucleation or may have been excluded during processing and sectioning. More often observed have been the exoskeletal, respiratory and reproductive structures, the head in particular was rarely found [13]. Also lacking in our samples was the surrounding host tissue, given that the lesions were not surgically resected.

On physical examination, a hypertrophied female sand flea and the tissue reaction around it may resemble arthropod bites, tissue growths, acariasis, cutaneous helminthiasis and cutaneous myiasis. In cordylobiasis and dermatobiasis the larva's respiratory punctum may look like the ano-genital-respiratory meatus of *Tunga*. Histology section shows a cystic body with cuticle and a haemocoel containing muscle and digestive structures however, unlike tungiasis there are neither ovaries nor ova [14]. Furthermore, the cuticle of the dipterous larva has thorn-like spines extending from the surface which are not seen in tungiasis [15]. With a tick-bite, remnant mouth parts may become enclosed by a down-growth of the epidermis and sclerotised fragments may be seen in the inflamed dermis [16]. In addition, sections with the acarid still attached may show digestive and other internal organs however, unlike tungiasis the haemocoel is outside the host's epidermis [17]. In scabies caused by a smaller acarid, the mites are more often found in the stratum corneum than in the dermis where *Tunga* dwells [18]. Histology section through the skin burrow shows a cystic structure with internal organs and a dorsal cuticle with minute spines; the latter is not characteristic of *Tunga* [19,20].

Conclusion

While the external appearance of the hypertrophied female sand flea was easily recognisable, the morphological detail was essential for the exclusion of other parasitoses. The exoskeletal, digestive, reproductive and respiratory structures demonstrated were distinguished from those seen in cutaneous myiasis, acariasis and cutaneous helminthiasis. The organisms were then reported as tissue-embedding haematophagous oviparous arthropods of the genus *Tunga*. Tungiasis has since been listed among the thirteen neglected tropical diseases of highest public health importance in Uganda.

Acknowledgement

We thank the Director General of Health Services, Uganda Ministry of Health for permission to publish this article. Ms Ruth Nakigudde of the Department of Pathology, School of Biomedical Sciences, College of Health Sciences, Makerere University processed the tissues and prepared the histology slides.

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How to cite this article: Elizabeth Sentongo, Samuel Kalungi and George Mukone. Histological Demonstration of the Organisms Causing Human Tungiasis in Eastern Uganda. J Cytol Histol 10 (2020) doi: 10.37421/jch.2020.11.551